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(72) RICHARDSON, Timothy M., CA
(71) NORTHERN EDGE ASSOCIATES INC., CA

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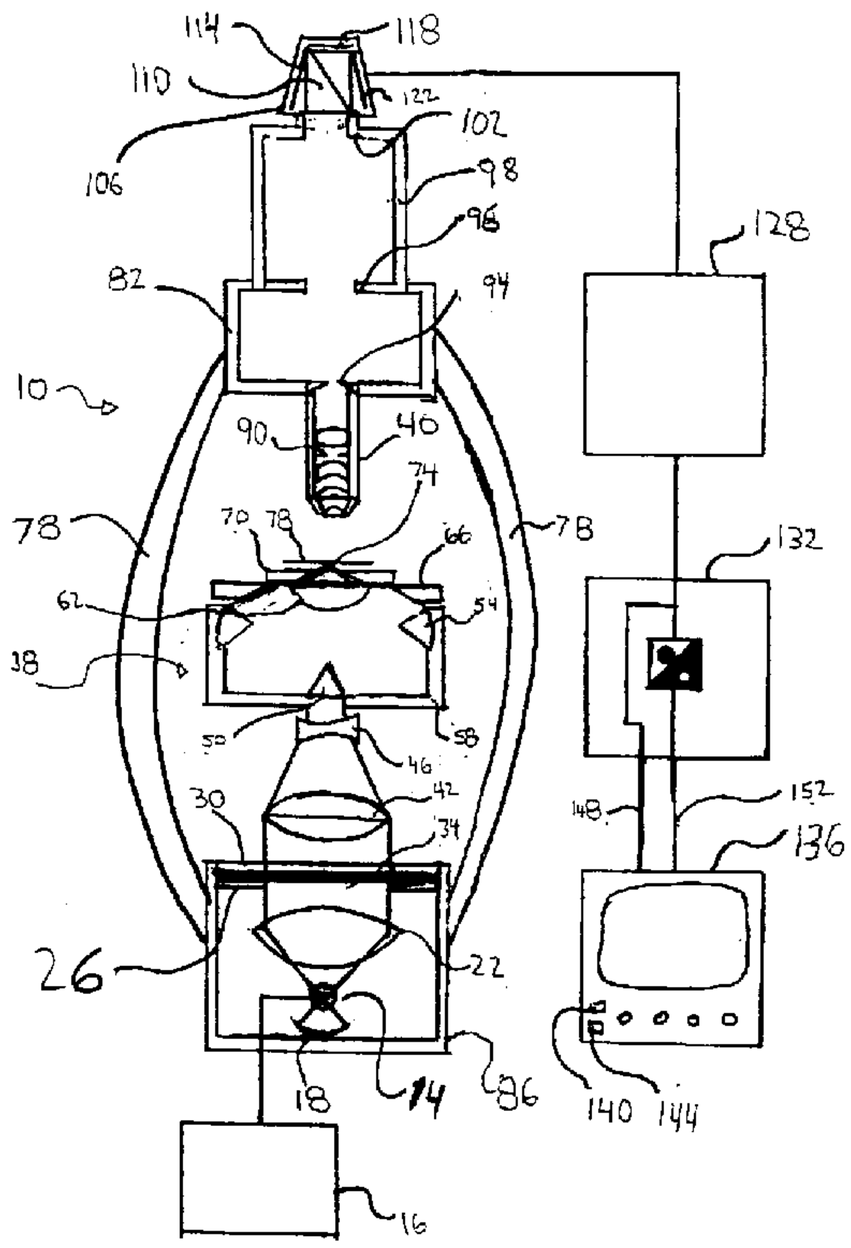
(54) **ULTRAMICROSCOPE A CONTRASTE INVERSE ET METHODE**

(54) **INVERTED DARKFIELD CONTRAST MICROSCOPE AND METHOD**



(57) A novel microscope and method of obtaining images includes a combination of the conventional darkfield illuminations technique with electronic image inversion (converting a positive to a negative image) and other improvements to further enhance the contrast and resolution of the final image. The microscope and method are referred to herein as Inverted Darkfield Contrast (IDC) and are believed to be particularly suitable for viewing live cells in real time with no staining or preparation.

ABSTRACT

A novel microscope and method of obtaining images includes a combination of the conventional darkfield illuminations technique with electronic image inversion (converting a positive to a negative image) and other improvements to further enhance the contrast and resolution of the final image. The microscope and method are referred to herein as Inverted Darkfield Contrast (IDC) and are believed to be particularly suitable for viewing live cells in real time with no staining or preparation.

INVERTED DARKFIELD CONTRAST MICROSCOPE AND METHOD

FIELD OF THE INVENTION

The present invention relates to microscopes and methods of obtaining images therewith. More particularly, the present invention relates to a method of obtaining images with Inverted Darkfield Contrast (IDC) microscopes and a novel IDC microscope.

BACKGROUND OF THE INVENTION

For many years light microscopes have been considered a mature technology. While there have been notable attempts to extend the capabilities of the light microscope, to date such attempts have not achieved substantial gains in performance and have generally been obtained at significantly increased costs.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a novel IDC microscope and a novel method of obtaining images with an IDC.

According to a first aspect of the present invention, there is provided a A novel method of achieving contrast for microscopical imaging of preparations of living cells and other types of objects is described along with improvements to microscopes. This method combines the traditional darkfield illumination technique with electronic image inversion (converting a positive to a negative image) and other improvements to further enhance the contrast and resolution of the final image. The method is referred to herein as Inverted Darkfield Contrast and is believed to be particularly suitable for viewing live cells in real time with no staining or preparation.

The embodiments shown herein are primarily based on a video microscope in which image resolution, contrast and optical efficiency are optimized. In microscopes in accordance with the present invention there is usually no intervening binocular or trinocular arrangement or eyepiece between the objective and the imaging system, which can be any

type of imaging means including film cameras, analog or digital video cameras or image intensifiers. The microscope system can use a pre-focused and aligned lamp and reflector to direct a larger than usual portion of the light from the lamp into the illuminating beam. The illuminating beam is directed through a beam expander which controls the diameter of the illuminating beam while maintaining parallel rays of light. The illuminating beam passes through apertures to control stray light.

Careful attention is paid to controlling the illuminating wavelengths of light to improve the resolution of the microscope. In particular all the non-visible wavelengths in the ultraviolet (UV) and infrared (IR) portion of the spectrum are preferably eliminated to improve image quality. The rays of light leaving the objective are also passed through apertures and baffle tube(s) to reduce stray light and enhance contrast. Anti-vibration means are also provided to control the motion of the objective, relative to the sample being viewed, and the position of the imaging device relative to the objective. Control of stray light in the objective and in the coupler between the objective and the imaging device also help to improve contrast and resolution.

The signal from the imaging device is inverted to form the negative of the normal image. In this way the traditional darkfield image appears as a high contrast brightfield image in the final monitor or computer display.

The present invention comprises a variety of mechanical and optical improvements to a microscope in order to achieve Inverted Darkfield Contrast (IDC). More specifically, a video microscope is provided which can include improvements to the illumination system, the condenser, the slide holder, the objectives, the tube, the microscope stand and the image acquiring system to produce a novel IDC microscope.

The present invention provides a method for obtaining high contrast images of living biological samples such as cells in real time with no staining or fluorochemistry required. The method is applicable to imaging a variety of materials, substances and structures, including cells, internal cellular structures, bacteria, viruses, fungi and plant

materials. The present invention also includes improvements in microscope technology including improvements to stand design, illuminators, condensers, objectives, imaging systems and to video processing.

While the concept of darkfield imaging is not new and the use of video positive to negative inversion is known in the television broadcast special effects field, the present invention is the first application of these unrelated techniques to obtain high contrast images of samples such as living biological material. The present invention provides particular advantages as can provide images which look like stained biological materials, so that biologist can readily interpret and accept the information that the images present, without requiring the staining of the imaged samples. The present invention can improve the contrast, resolution and speed of the image, without significantly increasing the cost or complexity of the microscope.

BRIEF DESCRIPTION OF THE DRAWING

Preferred embodiments of the present invention will now be described, by way of example only, with reference to the attached Figure, wherein:

Figure 1 shows an embodiment of an IDC microscope in accordance with the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention forms a darkfield image with a high numerical aperture (NA) optical system and electronic inverts the digitized darkfield image to produce a negative image of the darkfield image. The negative image is an apparent brightfield image, with very high contrast.

While it is possible to implement the method of the present invention using standard microscope illuminators, it is presently preferred that the light source employed with the present invention be considered on the basis of a "photon budget", where the destination of each photon from the light source is mapped and accounted for in the design of the IDC microscope. In order to achieve this goal, in a presently preferred embodiment of the

invention, the light source is selected and constructed as follows.

In conventional microscopes, tungsten, tungsten halogen, quartz halogen, or arc light sources are employed. These light sources are not well controlled in terms of the position of the light emitting surface of the source and consequently there is usually a means for centering the light emitting surface in the X, Y and Z directions with respect to the optical path of the microscope.

In contrast, in the presently preferred embodiment of the invention illustrated in Figure 1, the microscope indicated generally at 10, can employ a light source 14 in which the exact location of the light emitting component is exactly controlled by the body of light source 14. This eliminates the need for a centering mechanism for light source 14 and ensures that substantially the highest possible intensity and geometrical control of the beam and repeatability is achieved. Suitable examples of such light sources are the Welch Allen lamps for medical applications, the ILC arc lamps, the GE and Sylvania prefocused lamps and other, similar, light sources. To the best of the present inventor's knowledge, to date these light sources have not been employed with light microscopes.

In microscope 10, light source 14, which is supplied with the necessary power from a suitable power supply 16, is mounted such that as much light as possible from the light emitting surface, or surfaces, is focused by a suitable illuminator focus means, such as a mirror 18 behind light source 14 and/or a lens 22 in front of light source 14. The light from the back of light source 14 is focused back onto the emitting surface(s) of light source 14 by mirror 18. The light from the front of light source 14 and that returned by mirror 18 is focused into a collimated beam by lens 22, or a set of lenses, in front of light source 22. Suitable apertures 26, baffles 30 or tubular structures (not shown) are employed to ensure that the light from lens 22 is substantially completely collimated. It is desired to collimate the light from lens 22 so that little or no off-axis light enters the condenser system, described below, of microscope 10. Such off-axis light would become "stray light" in the imaging optics and would degrade the contrast of the final image.

As most light sources emit light which is outside the range of human vision, and the corrected range of microscope optics, a filter means 34 is provided in the path of the illuminating beam to filter the light to correspond as closely as possible to the range of wavelengths for which the optics of microscope 10 are designed. Filter means 34 can be included anywhere in the illumination beam path between the light source 14 and the final optics of the condenser 38 and filter means 34 can consist of the one or more heat filters such as Schott KG1 or KG5 glass, can include additional interference filters to attenuate the red or blue end of the light spectrum, and can exclude the ultraviolet portion of the spectrum with Schott WG or GG series filters.

By restricting the wavelengths of light present in the illuminating beam it is possible to operate the objective 40 of microscope 10 with light which forms a higher resolution image of the object due to the matching of the light to the design specifications of objective 40 of microscope 10.

Where it is desirable to include fluorescence capability for microscope 10, a position can be provided in filter means 34 for an illuminating filter which limits the illuminating beam wavelengths to only those wavelengths that are important for exciting the fluorophores being used with the sample. In this case, the substrate of this filter should be kept as thin a possible so that the ray path of the illuminating beam is disrupted as little as possible. As the method of illumination in the present invention is darkfield, fluorescent imaging can be applied to this method with almost the same quality of results as with reflected light microscopy, even though this is nominally a "brightfield" technique.

In order to match the illuminating beam to characteristics of condenser 38 being employed, additional optical systems are included. Specifically, the collimated illumination beam from light source 14 passes through one or both of a two types of optical systems. The first type of optical system is operable to modify the illuminating beam dimension to match the optical requirements of condenser 38. This system can be a system of fixed lenses 42 and 46 or a zoom lens device (not shown), either of which operate to supply substantially the highest possible amount of light from illumination source 14 to condenser

38 in a beam geometry selected to take full advantage of the characteristics of condenser 38.

When a darkfield condenser is employed, a parallel beam of light can be the most advantageous while in a conventional brightfield condenser, a converging beam of light is desired where the converging beam presents the image of the filament of the lamp at the back focal plane of the condenser to achieve Kohler illumination. If desired, the illumination beam can pass through a second optical system (not shown) to reshape the illuminating beam to achieve Kohler illumination, as is well known in the art.

Condenser 38 can be a high numerical aperture darkfield condenser of any type, as is known to those of skill in the art. The design of condenser 38 should create an inner and outer cone of illumination of numerical aperture to match the optical characteristics of the objective 40 being employed. The presently preferred numerical apertures for condenser 38 are 1.27 for the inner cone and 1.33 for the outer cone.

A numerical aperture of 1.27 is presently preferred for the inner cone so that objectives of 1.25 numerical aperture can be used without requiring additional stops or irises to control their numerical aperture and the contrast of the darkfield effect. A numerical aperture of 1.33 is presently preferred for the outer cone to match the index of refraction of aqueous media. As will be apparent to those of skill in the art, for higher index media or for highlighting high index materials that are directly in contact with the slide, then it can be preferred to employ in condenser 38 an aperture of 1.4 for the outer cone. For "extreme" applications, and where the characteristics of the media surrounding the sample and the sample itself allow, it is presently preferred to employ a condenser 38 with a numerical aperture of 1.42 for the inner cone and of 1.47 or higher for the outer cone.

The reason to reduce the numerical aperture of the outer illuminating cone in aqueous applications is to limit the stray light which results when a portion of the illuminating cone from condenser 38 is reflected by total internal reflection at the glass-water interface of the sample back into condenser 38 where it becomes stray light. Alternatively, returning stray light can be trapped and absorbed in light traps or dumps created by suitably

baffled or design surface geometries.

The presently preferred types of designs for condenser 38 include the Zeiss ultra-dark field condenser, the older design Leitz darkfield condenser for the oil immersion use, or the current production LOMO high numerical aperture darkfield condenser with an inner NA of 1.2.

It is presently preferred that condenser 38 employ the cone darkfield illuminator, or the coaxial darkfield/brightfield illuminator, both of which were designed after the work of the J. E. Barnard, circa 1933 and 1925 respectively and which are described in various papers and publications. In particular, in the cone condenser illustrated in Figure 1, the illumination beam passes through a conical prism 50 which forms an angled, but still collimated, annulus of light. This annulus is reflected off the surface of a circular mirror ring 54 which focuses the light to a hollow cone of the desired geometry. The elements of condenser 38 are contained in a suitable housing 58.

The illumination beam leaving condenser 38 passes through a spherical lens 62 in such a way that the rays from the surface of mirror ring 54 pass through the surface of lens 62 at right angles and are undeviated. As condenser 38 is achromatic, it can be employed equally well for infrared, visible or ultraviolet light imaging applications.

The illumination beam from condenser 38 passes through the stage 66 of microscope 10 and the slide 70 which supports the sample/object 74 being imaged. In most circumstances, sample 74 will be covered by a cover slip 78. Due to the high numerical apertures employed, condenser 38 is preferably connected to slide 70 by a film of immersion oil as is well know to those of skill in the art.

Microscopes have historically been constructed with C-shaped frames with the objective and eye-piece at the upper end of the frame and light source and stage at the lower end of the frame. The present inventor has determined that, while convenient to use and manufacture, conventional C-shaped frames suffer from disadvantages in that these frames

are susceptible to undesired vibrations, and in fact are shaped and surprisingly act much like tuning forks. It has been found that external vibrations from any source and of virtually any frequency tend to excite the tuning fork shape of the conventional C-shaped frame to vibrate at its own resonant frequency and this can distort the image resolved by the microscope. These disadvantages are particularly exacerbated with the present invention which otherwise can allow microscope 10 to resolve objects as small 250 nanometers, or less, and to detect objects as small as less than 50 nanometers. Accordingly, it is preferred to attenuate vibration of the microscope frame such that undesired movement of objective 40 relative to the sample 74 being imaged is inhibited.

The present inventor has determined two approaches to attenuating or eliminating this undesired vibration. The presently preferred first approach is to include or add new wing shaped braces 78 which connect the head of the microscope 82 to the base 86 of microscope 10. Braces 78 are attached to microscope 10 along the vertical optical axis and on either side of stage 66 of microscope 10. Braces 78 can be fabricated or cast and are preferably made of a material, such as steel, which has a relatively low elasticity and tendency to vibrate. Preferably, braces 78 are designed to have as little resonant vibration as possible and, of the vibration which can not be eliminated, braces 78 are designed such that their resonant frequency is not a harmonic or sub-harmonic of the fundamental frequency of the vibration of the microscope C-shaped frame. In this manner the vibration of each of the frame and braces 78 tend to damp the vibrations of the other.

Another approach is to employ a tubular design for the frame of microscope 10, wherein the tube surrounds stage 66 of microscope 10 in much the same way as the design of conventional electron microscope chambers and columns. Such a tubular design can virtually eliminate the Z axis vibrations of objective 40 relative to the sample 74.

Objective 40 of microscope 10 can be designed as a fixed focal length objective to produce a completely corrected image at the first image plane of objective 40. Furthermore, objective 40 preferably is designed such that any stray light from sample 74 which is not to form an in-focus part of the final image is attenuated by stops, irises or

geometrical light trapping means. The use of an aperture 90 or an adjustable iris (not shown) in the same location, is desirable to exactly match the illumination beam to the numerical aperture of objective 40 to ensure that the best possible darkfield image is obtained.

It is presently preferred to employ an adjustable, rather than fixed, iris in microscope 10 as the opening of such an adjustable iris to allow objective 40 to operate at an aperture greater than the inner illuminating cone of condenser 38 allows microscope 10 to be employed in an unusual brightfield mode which accentuates surface topography of sample 74 while substantially maintaining a high contrast of the obtained image. Further, a slight enhancement of the resolving power of objective 40 can be obtained due to the increase in numerical aperture. This brightfield mode can provide a novel image appearance which can provide image information which was previously unobtainable. If objective 40 is an infinity corrected objective, then a suitable matching tube lens (not shown) is employed to convert the infinity light to fixed focal length light.

If a fluorescence capability is to be provided for microscope 10 and an infinity corrected objective 40 and matching tube lens is employed in microscope 10, an emission filter (not shown) or filters for fluorescence microscopy can be located between objective 40 and the tube lens in the infinity space. If a fixed focal length objective 40 is employed, then the emission filter or filters for fluorescence microscopy can be included in head 82 of microscope 10.

Where this emission filter (or filters) is used with fixed focal length objectives, it is preferred to coat the emission filter onto the thinnest possible filter substrate so that the deviations of the image due to the index of refraction of the filter coatings and substrate will be as small as possible. The filter or filters can be on a slide or can be on a turret or filter wheel arrangement, as will be apparent to those of skill in the art. Where these filters are used to create artificial color, when a monochrome digital camera is employed are where they are used for multiple fluorescence techniques or for numerically processed pseudo-color computer driven imaging is employed, then the filter turret or wheel can be digitally controlled and electrically driven.

A fixed focal length objective can be expected to produce a brighter (more photon efficient) and a more highly corrected image in the first image plane than an infinity corrected system due to the lower number of surfaces and components relative to an infinity focused objective. Where fixed focal length objectives are employed, it can be desirable to design the objectives with a much shorter back focal length in order to substantially reduce the overall height of the microscope.

Microscope 10 can include a single objective 40, or can include two or more objectives 40 which can be selected for use as desired. In this latter case, the objectives 40 can be mounted on any appropriate mounting means, such as the conventional revolving nosepiece used in many microscope designs.

The light leaving objective 40 passes through a first aperture 94 and then, when leaving head 82 of microscope 10, through a carefully controlled second aperture 96 which blocks any light rays not in the desired image forming beam. The walls of head 82 and a coupler 98 are of relatively large internal diameter to further reduce stray light and improve image contrast. The inner surfaces of head 82 and coupler 98 can also be machined with geometrical surfaces to control and substantially eliminate light reflections from reaching the imaging means, discussed below.

The inner surfaces of the objective 40, head 82 and coupler 98 are preferably finished in a flat black or other suitable finish to obtain the lowest possible coefficient of reflection for light of the wavelengths being employed to form the final image. Generally this will be flat black or anodized black finishes.

Before the light containing the image information reaches the imaging means, it passes through another aperture or stop 102 which is shaped to further limit stray light. This aperture can be a square or other shaped aperture to match the geometry of the imaging means.

In the embodiment shown in Figure 1, the imaging means 106 is a three

detector CCD camera with an internal prism 110 and three charge coupled array detectors 114, 118 and 122 is placed at the primary image plane of the objective (or the objective tube lens combination in the case of infinity corrected optical systems). Placing imaging means 106 in the first focal plane of the objective is presently believed to be advantageous since it improves the image brightness, as otherwise the presence of any intervening optics would introduce light losses, and since it maintains the highest possible image resolution and contrast which would otherwise be degraded by any other intervening optics.

The electronic image acquired by imaging means 106 is provided to a control unit 128 which can contain automatic gain controls, white balance, black balance and auto iris functions, all of which can be controlled or limited by the operator of the system for maximum imaging control and flexibility.

The electronic signal from imaging means 106 and control unit 128 is then provided to image inverter system 132 which electronically converts it to obtain either the luminance or luminance and chrominance negative image of the image provided to it from control unit 128. A trivial example of the function of image inverter system 132 would be that an image of a black spot on a white background is converted to a white spot on a black background. Both control unit 128 and the image inverter system 132 can be included as internal or integral components of the imaging means 106.

Depending on how the chrominance information is to be handled by the image inverter system 132, then the resulting color in the final image leaving image inverter system 132 can either be color correct image or a color negative image of the image provided to system 128. Further, depending on what type of images are coming from sample 74, it can be desirable to view the image without the inverting conversion being performed. Accordingly, image inverter system 132 can forward both the inverted image and the non-inverted image. In this way, image inverter system 132 provides as many as three modes of output images. The first mode being the positive image, the second mode being the negative image with color in the negative and the third mode wherein color is not in the negative, but the brightness is the negative.

In the embodiment of Figure 1, image inverter system 132 does nothing to alter the resolution or the contrast of the image. The resolution and the contrast is solely derived from the optical methods employed being darkfield illumination, optimal correction of the optics to provide superior image quality and photon efficiency, and careful attention to photon budgets to account for substantially all the photons leaving light source 14 to ensure that they contribute to the final in-focus image. Control of stray light in microscope 10 is an important factor in obtaining the final high contrast image.

Alternately image inverter system 132 can be implemented in a computer-based image processing system (not shown) wherein the imaging means is an analog camera and the computer contains an image capture card for converting the analog to a digital image or where the imaging means is a digital camera and the computer processes the digital data directly. The advantage of using the computer-based image processing system with this type of microscopy is that color can be mapped to the acquired image in a defined way to best suit the application. Contrast expansion and pseudo-color techniques, along with other known image processing techniques, can be beneficial to extract further information from the images obtained.

The final acquired and processed image is displayed to the operator on a monitor 136 which can be an analog monitor or a computer monitor. It can be desirable to provide switches such as 140 and 144 to select the video mode being viewed. In the embodiment of Figure 1, switch 140 selects the positive video image provided via connection 148 and switch 36 selects the negative video image provided via connection 152.

The final image can be recorded using analog means such as on video tape or digitally as digital video or digital image files of either still (JPEG) or motion video (MPEG).

This method of microscopy can be used with high power high numerical aperture objectives or with lower power objectives. The main limiting factor is the numerical aperture of the objective so that the objective aperture is smaller than the inner illuminating

cone of the illumination system. This makes microscopes in accordance with the present invention ideal for examining cells, such as human biopsy or plant cells, at low magnifications initially and then switching to higher magnifications later on for more detailed analysis.

As will be apparent to those of skill in the art, it is possible to employ electrochemical, electroluminescent, fluorescent, liquid crystal or image intensifier based schemes to provide the positive to negative conversion in this method. If such means are used, then conventional binoculars or trinoculars can be used to view the image of the object but some of the contrast and resolution will be lost.

It is further contemplated that ultrafine focussing of the microscope can be accomplished by controlled distortion of the vibration control braces 78 of microscope 10. For example, if a hydraulic cylinder (not shown) is used to couple the braces 78 together, then adding fluid to the hydraulic cylinder will force the braces 78 apart and deflect objective 40 towards sample 74 very slightly, thus providing a very fine focus control. If a very fine screw (not shown) is used to drive a very small bore piston (not shown) into a cylinder (not shown) filled with hydraulic oil and the resulting pressurized oil is supplied to the hydraulic cylinder connecting braces 78, then a very ultrafine focus can be implemented. The same type of function can be accomplished with a screw mechanism either in tension or compression between the braces 78 so that adjustment of the screw mechanism accomplishes the fine focus.

It is also contemplated that microscope 10 can employ one or more piezoelectric struts (not shown) between braces 78 to accomplish the ultrafine focussing of the microscope. Variation of the voltage on the piezoelectric struts will shift the focus of the microscope slightly.

A further application of this piezoelectric system is to move the microscope Z adjustment in synchrony with a vibrating sample 74 to obtain images of samples undergoing or exhibiting fixed frequency vibrations.

It is possible that reflections from the surface of the CCD camera or other imaging means can bounce back and forth in the space between the CCD camera and the back lens of objective 40 or the tube lens. It is contemplated that a benefit can be obtained by inserting a photonic valve, such as a one way mirror, to eliminate light returning to the objective lens from the imaging means and thereby eliminate a possible source of stray light and to improve the contrast.

The overall size of microscope 10 can be substantially reduced by incorporating the light source 14 into the internal housing of the condenser 38. Such a design was proposed by Zeiss in the 1930 for darkfield condensers. If a brightfield and darkfield combination condenser such as the Barnard coaxial, described above, is employed then the size can be kept very small while maintaining both brightfield and IDC modes of operation.

In order to provide a low power and extremely robust and compact IDC microscope for field use in rough environments, one or more light emitting diodes (not shown) can be employed as the light source and a conical prism-type condenser can be employed to take best advantage of this type of illumination.

Where color correction is an important factor, an array of light emitting diodes of different wavelengths can be used with an LED control means to vary the relative brightness from each of the LED's. In this way a color matched lighting system can be obtained. Depending on how the LED's are arranged, the color and the position and style of illumination can be varied to meet the needs of the application.

The above-described embodiments of the invention are intended to be examples of the present invention and alterations and modifications may be effected thereto, by those of skill in the art, without departing from the scope of the invention which is defined solely by the claims appended hereto.

I claim:

1. A method for microscopy of living biological samples wherein a microscope with a high numerical aperture illuminating system is used with an imaging means to acquire an image which is then inverted to obtain a negative image so that the final image is displayed as a brightfield image.

2. A microscope in which the illuminating lamp incorporates means to substantially precisely locate the light emitting surface of the light source so that it will always be properly located with relation to the optics which it functions in relation to.

3. A microscope in which the lamp, mirror and collecting lens function to produce a substantially highly collimated beam of light with virtually no stray light.

4. A microscope which includes a lens or system of lenses between the collecting lens and the condenser which serve to change the diameter of the illuminating beam of the light so as to match the characteristics of the condenser.

5. A microscope which includes a holder for the slide in which the slide with immersion oil on the bottom of the slide can be slid into the optical axis of the microscope in such a way that the oil contacts the condenser while moving substantially in the Z direction only at the time of contacting and can then be removed from the optical axis in a reversed action.

6. A microscope which includes an objective lens where substantially all the aberrations have been corrected so that the image in the first focal plane is a superior image and the microscope does not require a second eyepiece to couple the image in the first focal plane to the imaging means.

7. A microscope where the vibrations of the "C" shaped frame of the microscope in the Z direction are reduced or eliminated by the inclusion of stiffening members on either side of the microscope which are placed in such a way as to damp Z axis vibrations.

8. A microscope where the image forming means which may be a CCD, digital camera or other electronic imaging means is placed at the first focal position of the objective lens.

9. A microscope where the image forming means which may be a CCD, digital camera or other electronic imaging means are placed at the first focal position of the tube lens in a infinity corrected system.

10. A microscope where an electronic circuit is used to convert the signal from the image forming means from a positive to negative image in order to convert darkfield to brightfield images and vice versa.

11. A microscope where a digital image processing system is used to convert the signal from the image forming means from a positive to negative image in order to convert darkfield to brightfield images and vice versa.

12. A microscope where a system of apertures, tubes of relatively large diameter and baffles are used to reduce stray light reaching the image forming means and thereby materially increase the contrast of the image.

13. A microscope where warping of the vibration damping means is used to accomplish ultrafine focussing of the microscope image.

14. A microscope where hydraulic positioning is used to accomplish the ultrafine focussing of the microscope image.

15. A microscope where one or more piezoelectric struts between the two vibration damping arms of the microscope are used to accomplish the ultrafine focussing of the microscope.

16. A microscope where piezoelectric struts between the two vibration damping arms of

the microscopes are used to move the microscope Z adjustment in synchrony with a vibrating object to obtain images of objects undergoing or exhibiting fixed frequency vibrations.

17. A microscope where there is a photonic valve which eliminates light returning to the objective lens from the image forming means and thereby eliminates a source of stray light and improves contrast.

18. A microscope where the light source is integrated into the condenser lens system.

19. A mirrored darkfield microscope where the light source is integrated into the condenser lens system.

20. A mirrored brightfield microscope where the light source is integrated into the condenser lens system.

21. A microscope where the objective lens has a short back focal length so that the image forming means can be placed at the first focal position of the objective lens where such focal position is much closer to the objective than conventional microscope systems.

22. A microscope where a ring of light emitting diodes is used as the light source and is placed inside a reflecting condenser system.

23. A microscope as in claim 22 where the light emitting diodes can be individually controlled to control the spectrum and position of the lighting in the microscope.

24. A microscope which includes a filter between the illuminating system and the condenser where such filter removes the infrared and or the ultraviolet portion of the illuminating light with a very high degree of efficiency in order to improve the image quality of the microscope.

25. A microscope with a filter as in claim 24 where such filter substantially limits the

illuminating light to the range of preferred chromatic correction of the objective lenses.

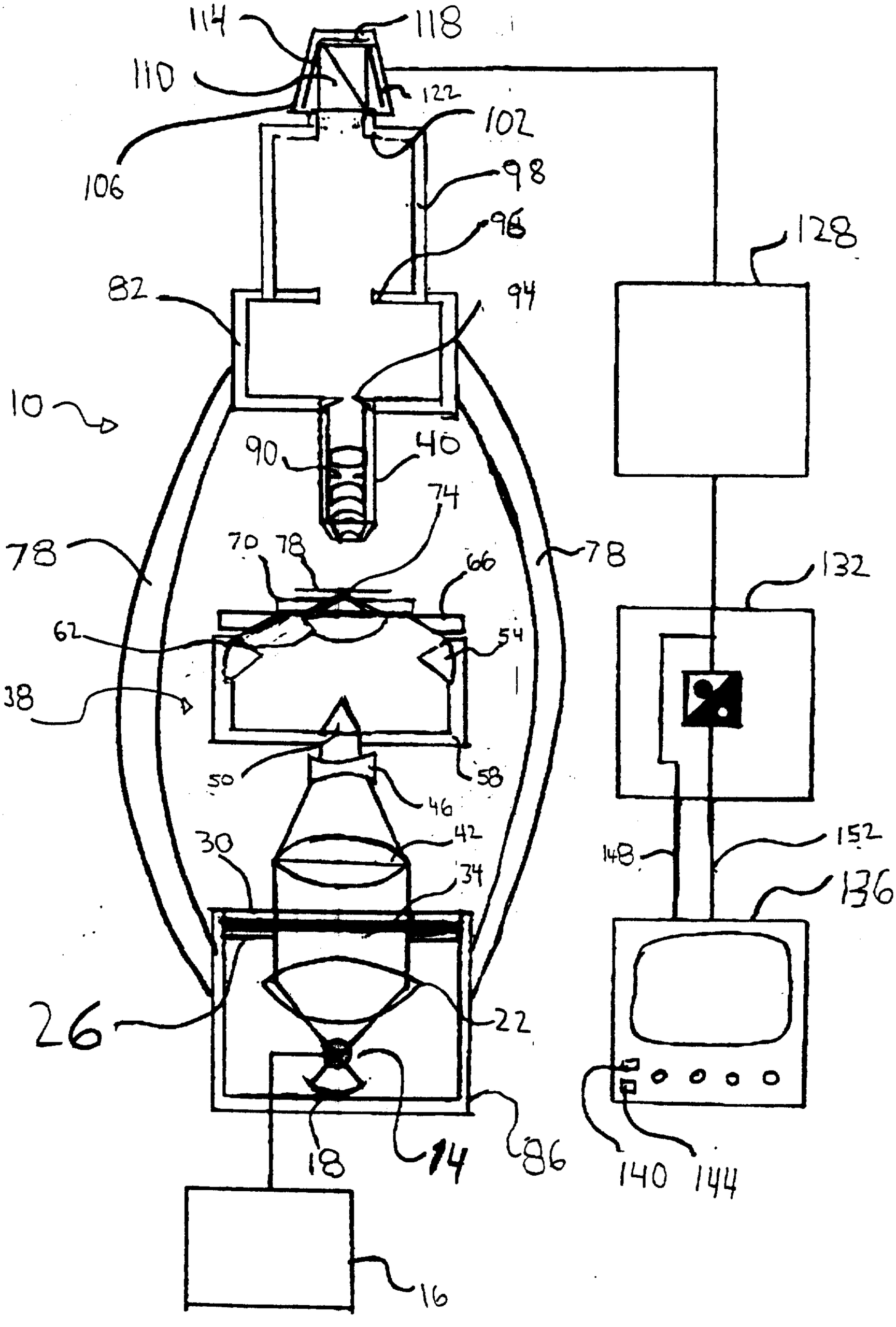

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FIGURE ONE